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Original Article

# Impact of obstructive sleep apnea on the 24-h metabolic hormone profile ☆

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ABSTRACT

*Objective:* Obstructive sleep apnea (OSA) has been associated with metabolic disorders. Sleep-disordered breathing could generate an altered rhythm in the expression of metabolic hormones, which could predispose to metabolic disorders. The aim of this study was to evaluate the effect of sleep apnea on diurnal variations in metabolic hormones.
*Methods:* Thirty-seven male, newly diagnosed, patients with OSA with an apnea–hypopnea index (AHI) $\geqslant$20/h and 11 male controls (AHI <10/h) matched for body mass index (±3 kg/m$^2$) were included. Six different samples were obtained from each subject during a period of 24 h. Levels of the metabolic hormones ghrelin, leptin, resistin, and adiponectin were measured in plasma by immunoassay.
*Results:* Patients with OSA (AHI (mean ± SD) 46 ± 26/h) were older than the controls (42 ± 9 vs 33 ± 9 years, $P = 0.01$). Differences in metabolic hormones between groups did not reach statistical significance at any point in the evaluation. No significant differences were observed in the area under the curve for any of the hormones analysed. Likewise, we did not detect diurnal variations in metabolic hormones.
*Conclusions:* The results of this study indicate that the day–night variations in the levels of several metabolic hormones are not influenced by the presence of sleep apnea.
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## 1. Introduction

Obstructive sleep apnea (OSA) is a common disease that affects 3–7% of the general population [1,2]. Sleep apnea is caused by the collapse of the upper airway during sleep, which leads to transient asphyxia. These events lead to a poor quality of life, as well as metabolic disturbances. The consequences of obstructive sleep apnea (OSA) are largely mediated by chronic intermittent hypoxia and sleep fragmentation, which might contribute to the pathogenesis of cardiovascular disease described in patients with sleep apnea [3]. Obesity, a major pathogenic factor in OSA in adults, is often present [4,5]. Furthermore, OSA-related factors contribute to the development of metabolic dysregulation: OSA has been associated with hormonal and metabolic alterations that could predispose patients to obesity. As obesity often coexists with OSA, it is not yet clear whether the presence of metabolic disorders is a consequence of OSA or simply reflects the effects of coexisting severe obesity [6].

Previous studies have explored the association between OSA and alterations in the secretion of metabolic hormones [7–9]. The methodological approach of these studies included individual samples obtained at one time point. It is of potential interest to explore the effect of OSA on metabolic hormone secretion during the night, which is when most of the changes associated with chronic intermittent hypoxia and sleep fragmentation are most apparent [10]. Moreover, changes during sleep may be partly related to circadian factors, as the central and peripheral circadian clocks have been linked to both whole-body and organ-specific energy metabolism [11]. The aim of this study was to evaluate the effect of sleep apnea on diurnal variations in metabolic hormones.

☆ This study was presented in abstract form, entitled 'Effect of sleep apnea on the 24-h metabolic hormones profile', at the American Thoracic Society Conference 2011, Denver, Colorado, USA, 13–18th May 2011.

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## 2. Methods

### 2.1. Subjects and ethics

We studied 37 male patients with OSA (apnea–hypopnea index (AHI) ⩾20/h) and 11 males without OSA (AHI <10/h) as a control group. All participants were referred by primary care physicians for evaluation of suspected OSA and were studied in the Sleep Unit of our institution. No participant had been previously diagnosed as having OSA. Each participant was interviewed and informed in detail about the purpose of this study. The subjects were matched for body mass index (BMI; ±3 kg/m$^2$) and waist circumference (±5 cm), and none of them had been involved in night-shift work or transmeridian travel, or had recently lost or put on weight. The diagnosis of OSA was established by full polysomnography (E-Series Compumedics, Abbotsford, Australia), including recording of oronasal flow and thoraco-abdominal movements, electrocardiography, sub-mental and pre-tibial electromyography, electro-oculography, electroencephalography, and transcutaneous measurement of arterial oxygen saturation ($SaO_2$). Apnea was defined as an absence of airflow for >10 s. Hypopnea was defined as any airflow reduction that lasts >10 s and results in arousal or oxygen desaturation. Desaturation was considered to be a decrease in $SaO_2$ ⩾4%. The AHI was defined as the sum of the number of apneas plus hypopneas per hour of sleep.

No participant suffered from any chronic disease (diabetes, hypertension, chronic obstructive pulmonary disease, liver cirrhosis, thyroid dysfunction, rheumatoid arthritis, chronic renal failure, and/or psychiatric disorders), or was taking any type of medication. The Ethics Committee of our institution approved the study, and all the participants signed their consent after being fully informed of its goal and characteristics. The analysis of day–night variations in the endothelial dysfunction markers and haemostatic factors of the subjects included in the present study has been previously published [12].

### 2.2. Twenty-four-hour repeated blood sampling protocol

The participants arrived at the sleep unit of our institution at 21:00, after fasting for ⩾6 h. They all received a 24 h standard isocaloric intake (2200 kcal) (Health and Human Services/US Department of Agriculture Dietary Guidelines 2010 for non-active men aged 31–50 years) to maintain the body weight registered on admission. The subjects ate four meals a day, distributed as shown in Fig. 1. They were exposed to light from 21:00h to 23:00h and from 7:00h to 18:00h and were studied in bed in the dark from 23:00h to 7:00h, as they slept. A heparinized venous catheter (Introcan Safety®; Braun, Melsungen, Germany) was inserted into an antecubital vein to allow serial blood sampling to take place throughout the night without disturbing sleep. Six different samples (20 mL each) were obtained from this catheter over the next 24 h (10:00, 02:00, 06:00, 10:00, 14:00, 18:00) (Fig. 1). Blood was collected in tubes containing EDTA (10 mL). The sample obtained at 10:00 was followed by an additional sample (10 mL) collected in tubes with no anticoagulant, for general biochemical assessment. Blood samples were immediately processed and centrifuged for 15 min at 2500 rpm (Jouan SA, model CR4 22, Saint-Herblain, France). Serum and plasma were frozen at −80 °C until analysis.

The participants remained in the hospital for the entire study. During the day, they were allowed to rest or to perform tasks involving little activity.

### 2.3. Biochemical analysis and enzyme-linked immunosorbent assays

Glucose, cholesterol, triglycerides and high-sensitivity C-reactive protein were measured in serum using standard automated enzymatic methods on a Hitachi 917 biochemical analyser (Roche Diagnostics, Indianapolis, IN, USA). High-density lipid (HDL) cholesterol was measured by a homogeneous, enzymatic colorimetric method, using a commercial reagent set (Roche Diagnostics). Low-density lipid (LDL) cholesterol concentration was calculated using the Friedewald equation. High-sensitivity C-reactive protein (hs-CRP) was measured using a chemiluminescent immunometric assay (Immulite 2000, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA). For hs-CRP the assay range was 0.2–100 mg/L, and the sensitivity was 0.2 mg/L. For mean levels of 0.85 mg/L the coefficients of variation (CV) were: 4.7% for CV intra-assay, and 7.1% for CV inter-assay. Resistin, total ghrelin (both intact and des-octanoyl forms), leptin, and adiponectin levels were determined in plasma EDTA by enzyme-linked immunosorbent assay, using commercially available kits. All the measurements were performed in duplicate and the mean values were used for analysis. For leptin (Millipore Corp., Saint Charles, MO, USA; Cat. #EZHL-80SK), the assay range was 0.5–100 ng/mL, and the sensitivity was 0.5 ng/mL. For mean levels of 11.26 ng/mL, the CV was 1.4% intra-assay, and 1.7% inter-assay. For ghrelin (Millipore Corp., Cat. #EZGRT-89K), the assay range was 50–5000 pg/mL, and the sensitivity was 50 pg/mL. For mean levels of 868.4 pg/mL, the CV was 1.11% intra-assay and 5.18 inter-assay. For adiponectin (Millipore Corp., Cat. #CYT350), the assay range was 100–6400 ng/mL and the sensitivity was 10 ng/mL. For mean levels of 5900 ng/mL the CV was 3.84% intra-assay and 5.5% inter-assay. For resistin (Invitrogen Corp., Camarillo, CA, USA; Cat. #KHP0051), the assay range was 2.5–20 ng/mL and the sensitivity was 0.1 ng/mL. For mean levels of 5.19 ng/mL the CV was 3.77% intra-assay and 6.8% inter-assay.

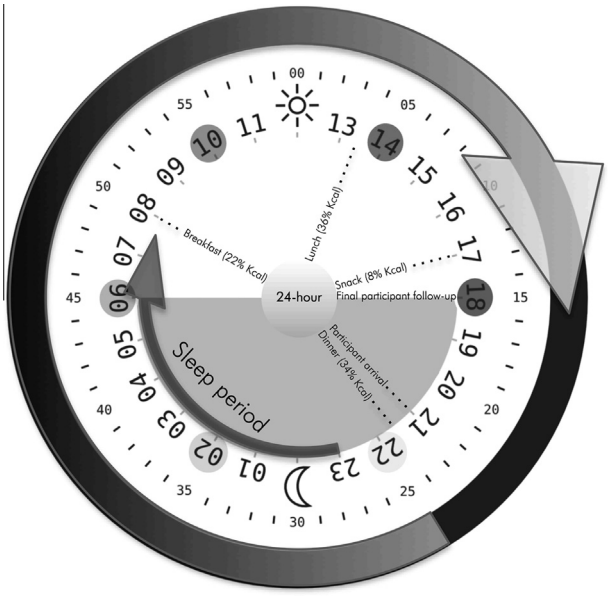


**Fig. 1.** Interventions for each patient in a 24 h cycle. For each study participant: 21:00h; participant arrives at sleep unit. 21:30h; dinner (34% calories). 22:00h; first blood sample collection. 23:00h-7:00h; sleep period. 14:00h; second blood sample collection. 18:00h; third blood sample collection. 8:00h; breakfast (22% calories). 10:00h; fourth blood sample collection. 13:30h; lunch (36% calories). 14:00h; fifth blood sample collection. 17:00h; snack (8% calories). 18:00h; Sixth blood sample collection. End of participant's follow-up. The patient leaves the hospital sleep unit.

### 2.4. Statistical analysis

The sample size was established with reference to similar studies in which metabolic hormone levels were evaluated but only at specific time-points, some studies being in populations with different characteristics to the population of this study [13–15]. The

number of subjects included in our study provided us enough statistical power (80%) to detect a significant difference (with a 5% level) in plasma levels of leptin, ghrelin, resistin, and adiponectin between OSA and controls greater than 2-, 1.68-, 2.13- and 1.82-fold, respectively. The differences in anthropometric and clinical characteristics between the patients with OSA and the controls were assessed using a non-parametric Mann–Whitney test. To evaluate the effect of OSA over the 24 h secretion of each metabolic hormone analysed, a Mann–Whitney test was used to explore the differences between the OSA and control patients at each time-point, along with the area under the curve (AUC), which was computed for each individual [16]. Linear mixed models were fitted for each hormone, in order to take into account within-individual variation caused by the longitudinal structure of the data. Different models performed to assess the effect of OSA alone and its joint effect on other variables, adjusting for any possible confusing effects or collinearity associated with OSA. We used the trapezoidal rule for the AUC computation, considering the 24 h period and separately for two sub-periods: night-time (22:00–10:00) and daytime (10:00–18:00). Spearman correlations were computed to evaluate the relationship between the hormone profiles and parameters related to OSA (AHI, arousal index, mean and minimal $SaO_2$). The threshold for statistical significance was set at 0.05. Bonferroni correction for multiple testing was applied when appropriate. Statistical analysis was performed using R software (R version 2.15).

## 3. Results

Table 1 shows the main anthropometric and clinical characteristics, as well as the biochemical parameters of the group of patients and controls studied. By definition, patients with OSA showed abnormal sleep parameters, whereas these variables were normal in the controls. Both groups of patients and controls presented similar BMI, waist circumference, systolic and diastolic pressure, Epworth Sleepiness Scale score, glucose, percentage of current smokers, HDL cholesterol, and triglycerides, although the latter were slightly younger. Nevertheless, the age difference was lower in absolute terms, but is probably of marginal biological significance, as no significant correlations were found between adipokines and age in any patient (Bonferroni-adjusted: leptin, $P = 0.2$; ghrelin, $P = 1$; adiponectin, $P = 1$; resistin, $P = 0.9$). OSA was severe in the patient group, as assessed by AHI, and the patients presented greater neck circumference, total cholesterol, LDL cholesterol, and C-reactive protein than the control group.

**Table 1**
Anthropometric, clinical, and biochemical variables in patients with obstructive sleep apnea (OSA) and control subjects.

| | OSA patients ($n = 37$) | Controls ($n = 11$) | $P$-value |
|---|---|---|---|
| Age (years) | 42.1 (9) | 33.4 (9) | 0.012 |
| BMI ($kg/m^2$) | 28.3 (4) | 26.4 (5.1) | 0.28 |
| SBP (mmHg) | 129.7 (12.5) | 125.3 (14.2) | 0.36 |
| DBP (mmHg) | 79.6 (12.5) | 73.8 (8.6) | 0.09 |
| Smokers, $n$ (%) | 10 (27) | 5 (41) | 0.37 |
| Neck circumference (cm) | 41.4 (2.6) | 38.6 (2.9) | 0.022 |
| Waist circumference (cm) | 103.3 (12.1) | 94.5 (14.1) | 0.17 |
| ESS score | 8.2 (5.1) | 8.3 (5) | 0.96 |
| TST (min) | 350 (61) | 329 (48) | 0.16 |
| AHI ($h^{-1}$) | 46.1 (26.1) | 5.8 (3.6) | <0.0001 |
| Arousal index ($h^{-1}$) | 20.8 (25) | 1.07 (2.4) | 0.0003 |
| Mean $SaO_2$ (%) | 93.3 (3.5) | 96.3 (1.9) | 0.0043 |
| Minimal $SaO_2$ (%) | 81 (7.4) | 90.4 (2.9) | <0.0001 |
| Glucose (mg/dL) | 103 (18) | 97 (10) | 0.36 |
| Total cholesterol (mg/dL) | 184 (31) | 162 (26) | 0.028 |
| HDL cholesterol (mg/dL) | 43.6 (9.9) | 41.4 (11.4) | 0.73 |
| LDL cholesterol (mg/dL) | 114 (27) | 84.7 (10.4) | 0.009 |
| Triglycerides (mg/dL) | 144 (135) | 158 (67) | 0.42 |
| hsCRP (mg/L) | 0.26 (0.28) | 0.1 (0.18) | 0.003 |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ESS, epworth sleep scale, TST, total sleep time; AHI, apnea–hypopnea index; $SaO_2$, transcutaneous arterial blood oxygen saturation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein.
Values are mean (SD).

Table 2 shows the mean values for the plasma levels of hormones at each time-point, analysed over a 24 h period, in the group of patients with OSA and in the control group. Fig. 2 shows the diurnal evolution of the plasma levels of hormones observed in each group (linear model adjusted for age and neck circumference). Although all the markers, apart from adiponectin, tended to be slightly higher in patients with OSA, the differences did not reach statistical significance at any of the time points evaluated. There was, however, a remarkable although non-significant increase in the mean values in the leptin plasma levels of the OSA patient group, ranging from 49% to 108%, with respect to the controls. To further analyse the potential time-dependent differences, AUC values were computed and compared between both groups; there were no significant differences between the two groups (Table 2). Additionally, to evaluate the specific influence of nocturnal intermittent hypoxia on metabolic hormone profiles, a sub-analysis for AUC for daytime and night-time was performed, which found no significant difference between groups for any of these periods. Similar results were obtained when the control subjects were compared with OSA patients classified according to the severity of their disease (mild–moderate OSA (AHI 20–30/h) and severe OSA (AHI >30/h) groups) (data not shown). There were no positive correlations between metabolic hormone AUC values and sleep parameters (Table 3). Nevertheless, arousal index was negatively correlated with resistin. The mean and minimum $SaO_2$ were negatively correlated with leptin (Fig. 3).

## 4. Discussion

This study has explored the effect of OSA on the 24 h metabolic hormone profile, showing that day–night variations in ghrelin, leptin, resistin, and adiponectin are not significantly different between OSA patients and controls without OSA. The difference over the observed time-period was not statistically significant when the OSA patients' hormones were compared directly with those of the control group, either over the 24 h period or at each time-point analysed (Tables 1 and 2). The results therefore suggest that sleep apnea has no direct effect on the oscillations of these metabolic hormones over a 24 h period.

Metabolic hormones play a significant role in the regulation of local metabolic processes (autocrine and paracrine function). They also regulate systemic processes, displaying typical endocrine properties [17]. Increased circulating leptin, a marker of leptin resistance, frequently occurs in obesity and is independently associated with insulin resistance and cardiovascular disease [18,19]. A previous study reported that OSA only had an effect on leptin plasma levels in non-obese subjects, even though leptin plasma levels were primarily associated with obesity [7]. Nevertheless, another study found only an association between leptin plasma levels and obesity in OSA patients [6]. We found that OSA patients had a mean increase in their leptin plasma level of 74% (Table 2), but this did not reach statistical significance. Patel et al. [13] explored diurnal leptin rhythms in OSA, reporting that morning and evening leptin levels were strongly associated with AHI; however, these associations were explained by differences in obesity. These findings were consistent with several earlier studies. Schafer et al. [20] found in multivariate analysis that the elevated morning leptin concentrations in sleep apneics were completely explained by differences in percent body fat, waist:hip ratio, and subcutaneous neck fat. Similarly,

**Table 2**
Assessment of the evolution of plasma hormone levels during a 24 h period.[a]

| Hormones | AUC levels | | | AUC analysis | | | | | | Mixed linear model analysis | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | OSA patients ($n$ = 37) | Controls ($n$ = 11) | Ratio (OSA patients:controls) | 24 h | | Daytime period (10:00–18:00) | | Night-time period (22:00–10:00) | | $P$ | Adj. $P$ |
| | | | | $P$ | Adj. $P$ | $P$ | Adj. $P$ | $P$ | Adj. $P$ | | |
| Ghrelin (pg/mL/24 h) | 13.534 (7659) | 12.201 (6548) | 1.11 | 0.64 | 0.67 | 0.96 | 0.87 | 0.51 | 0.43 | 0.59 | 0.97 |
| Leptin (pg/mL/24 h) | 219 (183) | 126 (81) | 1.74 | 0.15 | 0.71 | 0.24 | 0.46 | 0.13 | 0.24 | 0.12 | 0.16 |
| Resistin (pg/mL/24 h) | 156 (140) | 142 (87) | 1.10 | 0.76 | 0.83 | 0.86 | 0.79 | 0.58 | 0.67 | 0.78 | 0.81 |
| Adiponectin (pg/mL/24 h) | 130.418 (88.486) | 123.001 (81.030) | 1.06 | 0.9 | 0.96 | 0.7 | 0.54 | 0.95 | 0.88 | 0.86 | 0.7 |

AUC; area under the curve; OSA, obstructive sleep apnea.
Values are mean (SD). Adjusted (Adj.) $P$-values by a linear model are adjusted by age and neck circumference.
[a] Results from the AUC and mixed linear model analyses.

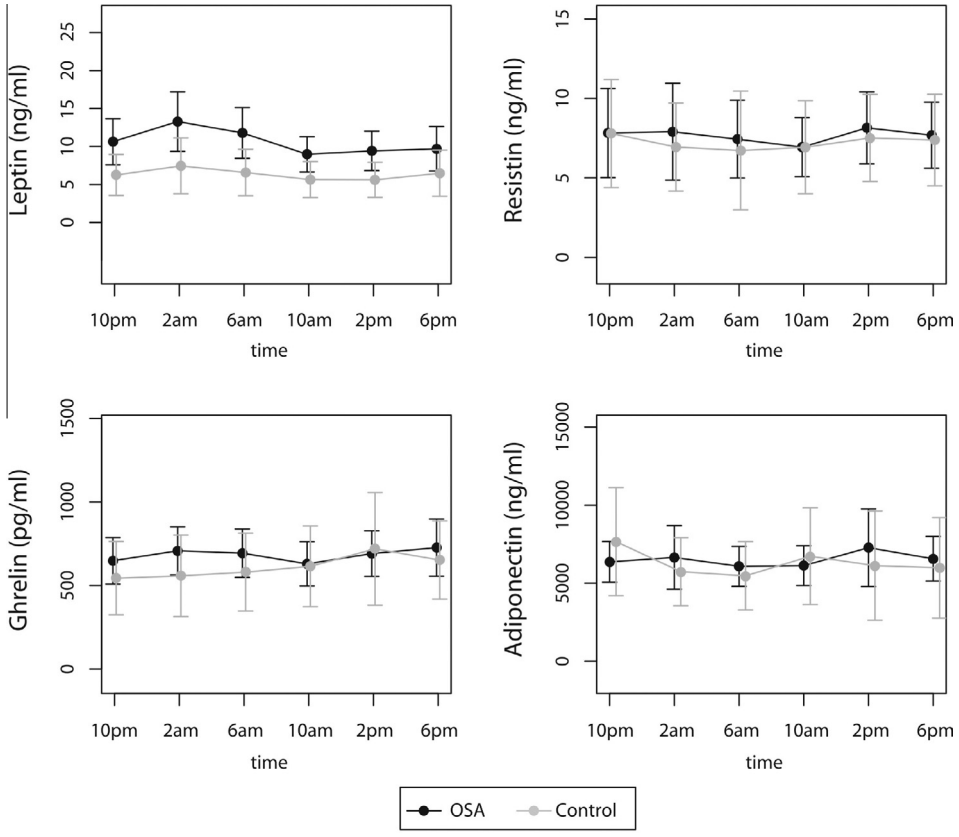


**Fig. 2.** Mean values of metabolic hormones at different times during the day in obstructive sleep apnea (OSA) patients and controls.

**Table 3**
Correlations between hormone AUC values and sleep parameters.

| | Apnea–hypopnea index | | | | | | Arousal index | | | | | | Mean $SaO_2$ | | | | | | Minimal $SaO_2$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24 h | | D (10:00–18:00) | | N (22:00–10:00) | | 24 h | | D (10:00–18:00) | | N (22:00–10:00) | | 24 h | | D (10:00–18:00) | | N (22:00–10:00) | | 24 h | | D (10:00–18:00) | | N (22:00–10:00) | |
| | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ |
| AUC | | | | | | | | | | | | | | | | | | | | | | | | |
| Ghrelin | −0.06 | 1 | −0.13 | 1 | −0.03 | 1 | 0.08 | 1 | 0.04 | 1 | 0.11 | 1 | 0.11 | 1 | 0.17 | 1 | 0.1 | 1 | −0.07 | 0.62 | −0.02 | 1 | −0.07 | 1 |
| Leptin | 0.25 | 1 | 0.22 | 1 | 0.27 | 0.96 | 0.3 | 0.64 | 0.27 | 1 | 0.32 | 0.48 | −0.5 | 0.004 | −0.49 | 0.006 | −0.49 | 0.006 | −0.44 | 0.03 | −0.44 | 0.03 | −0.44 | 0.03 |
| Resistin | −0.2 | 1 | −0.17 | 1 | −0.24 | 1 | −0.45 | 0.01 | −0.44 | 0.002 | −0.48 | 0.009 | −0.04 | 1 | −0.06 | 1 | 0 | 1 | 0.08 | 0.57 | 0.08 | 1 | 0.12 | 1 |
| Adiponectin | −0.06 | 1 | −0.08 | 1 | −0.04 | 1 | −0.13 | 1 | −0.14 | 1 | −0.12 | 1 | 0.09 | 1 | 0.08 | 1 | 0.06 | 1 | −0.01 | 0.95 | 0 | 1 | −0.02 | 1 |

$P$-values adjusted for multiple comparisons by Bonferroni method.

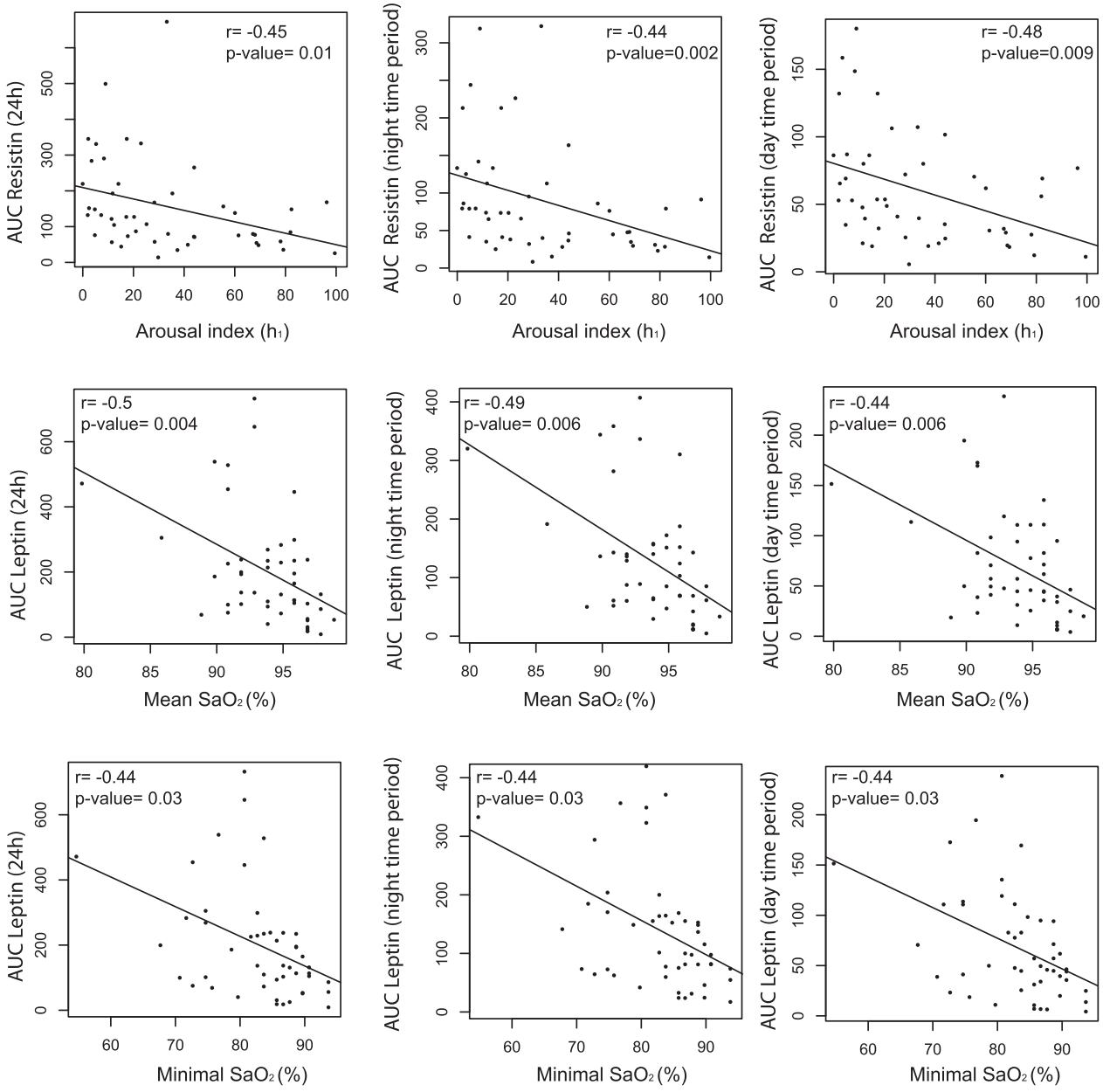


**Fig. 3.** Statistically significant correlations between hormone areas under the curve (AUC) and sleep parameters. $SaO_2$, transcutaneous arterial blood oxygen saturation.

Manzella et al. [21] found no association between leptin levels and OSA severity after controlling for BMI. Whereas our study found no significant correlation between leptin and AHI, there was a strong and significant correlation between leptin and BMI. Adiponectin is the most abundant adipokine and it has been implicated in the regulation of energy homeostasis, where it functions in combination with leptin [22]. It has been suggested that low baseline adiponectin levels play a role in the pathogenesis of cardiovascular disease associated with obesity [23,24]. Whereas some authors have described decreased adiponectin levels in OSA patients [25–27], others found no differences between OSA patients and controls [6,28,29]. Nakagawa et al. [30] described a nocturnal reduction in circulating adiponectin levels in severe OSA when compared with control subjects. Nevertheless, the present study found no diurnal variations in the plasma levels of adiponectin. This could be explained by the differences in the population analysed. Whereas Nakagawa et al. found a reduction in adiponectin levels in severe OSA patients with a BMI significantly higher that the control group, in our study OSA patients and controls presented similar BMI.

In the present study there were no diurnal variations in the plasma levels of ghrelin and resistin. Previous studies have described increased levels of ghrelin in OSA patients [9], although other authors found no differences between OSA patients and controls [6,31]. Similarly, there are contradictory results for resistin. Whereas some authors have shown that resistin levels rise in OSA patients [32], others have found decreased levels [33]. We found no differences between the groups, although it is plausible that OSA had a slight effect on plasma hormone levels. Plasma ghrelin level decreases after food intake. In the present study, blood samples at 22:00, 14:00 and 18:00 were collected in 1 h (or less) after food intake. Nevertheless it is important to consider that food intake by controls and patients was at the same times, and that samples were obtained at the same times, so this potential source of bias affected the two groups equally.

The main strength of our study resides in its design that allowed us to analyze the changes in metabolic hormone plasma levels during the sleep period, which is when apneic events occur. Moreover, the subjects included in the study were patients controlled for BMI and gender. Nevertheless, this study has several potential limitations. First, although patients with OSA and control subjects were matched for BMI, this did not exclude potential differences in body fat distribution; the OSA group had a greater neck circumference, and the results were adjusted for this variable. Second, the control subjects were slightly younger than the patients with OSA, although this difference was minor in absolute terms and is probably of marginal biological significance; the results were adjusted for this variable. For the subjects included in the present study, no significant correlation was found between plasma levels of the metabolic hormones with age. Third, we cannot exclude a type 2 error due to the relatively low number of control subjects included in the study. Nevertheless, sample size calculation for this study was performed based on previous studies that evaluated plasma levels of metabolic hormones, and which showed statistically significant differences in groups including a total number of subjects similar to that of the present study [15]. Fourth, our study population consisted of young overweight men, so we cannot extrapolate the results to other populations of OSA patients with different characteristics (women, obese patients, or elderly).

In conclusion, the results of this study indicate that the day–night variations in the levels of several metabolic hormones are not different between patients with sleep apnea and controls of similar weight.

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## Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.03.007.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sleep.2014.03.007.

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